"HORMONE" MEDIUM

A SIMPLE MEDIUM EMPLOYABLE AS A SUBSTITUTE FOR SERUM MEDIUM

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The investigations of many workers have long been directed toward the production of mediums that would answer the growth requirements of delicate organisms without the necessity of employing serous fluids — a complicated technic. The list of such mediums is long and many have answered the necessary requirements in part, while the work of English observers[1] in recent years, has pointed out the factors which are concerned in the reproduction of bacteria on artificial medium and in particular the part played by the so-called vitamins or hormones in establishing and conserving bacterial growth and the importance of the presence of suitable amino-acids.

Such mediums as they prepared are rather complicated and the ingredients are not always to be obtained, so that it seemed possible and necessary to attempt the production of mediums based on these considerations, but with such a simplified technic that it would be readily available under field conditions. Accordingly, various mediums were prepared, keeping the following factors in mind:

1. To extract the growth factors or "hormones" by bringing colloidal solutions, in this case melted agar or gelatin, into contact with the meat and blood.

2. To preserve these factors by limiting the amount of heating as much as possible, and most important, not to filter these solutions at any time through cloth, cotton or filter paper, since the "hormones" are readily adsorbed by vegetable fiber — one passage through filter paper of the finished product removing half of its initial growth value.

3. To furnish sufficient amino-acids by the use of a suitable peptone and the addition of egg-yolk.

4. To keep the hydrogen ion content in the proper zone, although this factor when the other conditions are suitable is not as important as has been assumed.

5. To so simplify the technic that the mediums could be produced under the most unfavorable conditions.

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[1] Lloyd: Jour. Path. and Bact., 1916, Vol. 21, Pt. I, p. 113; Cole and Lloyd: Ibid., 1917, Vol. 21, Pt. 2, p. 267.

Many mediums were prepared and various organs employed as a basis, and it was evident from the first that the beef usually employed and especially beef heart furnished sufficient "hormones" for the purpose, and that the question of getting suitable medium was merely that of a suitable technic.

The following method was finally developed as being most generally satisfactory:

"HORMONE" AGAR

	Gm.
Chopped beef heart or steak (must be comparatively fresh)	500
Water	1,000
Peptone (Bacto peptone gives the best results)	10
Agar (Bacto or thread agar that has been soaked)	16
Salt	5
Whole egg	1

All of these ingredients are placed in an ordinary enamel ware vessel, preferably a large coffee pot, and heated over an open flame with constant stirring until the red color of the meat infusion changes to brown, at a temperature about 68 C. Care should be taken not to run the temperature much above this point as the medium then begins to clot, which is undesirable at this time.

The medium is now titrated by the addition of normal sodium hydrate until it is slightly alkaline to litmus paper, and then 1 c c per liter is added in addition.

The vessel is covered and placed in the Arnold sterilizer or in a water bath at a temperature of 100 C. for 1 hour, removed, and the firm clot which has formed separated from the sides with a rod and the vessel returned to the sterilizer or water bath at 100 C. for 1½ hours.

It is now removed and allowed to stand at room temperature for about 10 minutes in a slightly inclined position; during this time the fluid portion separates and may be removed by pipetting or, in the case of the coffee pot, by simply pouring it off carefully. If it is poured through a fine wire sieve many small particles of meat clot may be caught.

The product is allowed to stand in tall cylinders for 15 to 20 minutes, until the fat present has risen to the surface where it can be removed. The medium is now tubed and sterilized by the intermittent method. Autoclaving is to be avoided.

If the medium, although usually clear enough for practical purposes, seems too turbid, further clearing may take place by filtration through glass wool, asbestos wool, sedimentation or centrifugation.

The product prepared according to the foregoing directions is available for all the usual laboratory uses and in addition has a growth value 10 times as great as standard agar and is at least as good as the average grade serum agar.

"HORMONE" SEMISOLID AGAR

Prepared as directed with the substitution of 5 gm. for 16 gm. of agar, this medium is tubed in about 10 c c amounts and employed for stab cultivations, and is an extremely useful medium both for

anaerobes and aerobes, and is especially suitable for the preservation of stock cultures. Experiments with the group of gram-negative cocci shows the meningococcus when sealed up in this medium and kept in the incubator to live for 3 months without transfer and the gonococcus for 2 months.

HORMONE GELATIN BROTH

Since an essential feature of the preparation of these mediums is the contact of colloidal solutions with the meat infusion the necessity arises of the addition of a small percentage of gelatin in the fluid medium. This is done by the addition of 10 gm. of gelatin as a substitute for the agar, otherwise the method is unchanged.

Such a medium is liquid at room and incubator temperatures and the presence of the gelatin in no way interferes with the usefulness of the product. It was feared that the gelatin might interfere with agglutination and precipitin tests for determination of type of the pneumococcus, but such proved not to be the case, the reactions being as sharp and prompt as in the medium usually employed for this purpose. Growth of practically all organisms tried is more rapid, more marked, and their peculiar characteristics are exaggerated, rendering tentative diagnoses easier to make. Capsule production by the pneumococcus is usually evident when inoculated from body fluids or organs rendering the use of serum medium unnecessary for this purpose.

MODIFICATIONS FOR SPECIAL PURPOSES

It was found that the "hormone" agar was as suitable for meningococcus contact cultivations as the mediums recommended for this purpose. Later, at the suggestion of Miss Anna Williams of the New York Health Department, the addition of small amounts (about 1 c c to the 100 c c) of defibrinated blood or better of laked blood (just enough to give a pinkish tinge to the poured plate) rendered the medium on actual test superior to glucose-ascitic agar as far as number of initial colonies established and size of colony are concerned, and has superseded other mediums for this type of work.

I have had the opportunity of testing the values of this modification in actual field work on many cases and in direct comparison to the standard mediums and have found, when experienced workers in this field are offered the opportunity of direct comparison of results, not to speak of the convenience the simplicity of preparation gives, that the decision is in favor of the newer medium. For general work

it may be employed successfully wherever ascitic agar would be indicated.

A modification of the "hormone" gelatin broth is by the addition of 0.15% dextrose and the addition of enough laked blood to give it a slight pink tint. This medium has remarkable growth value, the streptococcus showing a very distinct and marked growth in 4 hours, and in 18 hours a thick heavy mass of tangled chains at the bottom of the tube extending up about one-quarter of the height of the column of liquid, and when shaken up being as densely turbid as the growth usually seen on a 24-hour colon-broth culture.

When tried with spinal fluid from meningitis cases in which no organisms could be found in smears, distinct growth occurred in 9 hours, rendering a positive diagnosis possible. A peculiar feature of this growth is the fact that the organisms multiplied within the leukocytes transferred.

This medium is favorable for the growth of the pneumococcus, which gives a marked turbidity. Smears from the growth show well marked capsules.

Another modification, but not of such general utility, is the preparation of a substitute for Dorset's egg medium.

In this medium, which is prepared in a similar manner as the hormone agar, 1% aminoids are substituted for peptone and 5% glycerol is added to the finished product; the tubercle bacillus grows in 10 days almost as vigorously as on Dorset's egg medium.

Many other modifications and uses of this type of medium will doubtless suggest themselves and the fact that here is presented a simple utilization of several well known and demonstrated principles of bacterial growth, leads to the hope that other workers in this field will further improve on the methods.

SUMMARY

A basis for the production of laboratory mediums is given in which the points to be remembered are the extraction of the growth factors by colloidal solutions and avoidance of filtering by passage through any cloth, filter paper or cotton.

The advantages of medium so prepared are: Simplicity of preparation technic; great increase in growth efficiency; prolonged life of stock cultures, and convenience in the preparation of bacterial antigens, there being no serum element to be taken into consideration.